A copy of the Notification of May 1, 2001, with the Error Report included with the same are attached and a copy of the Notification to Comply dated May 1, 2001, is attached.

No new matter has been added. A Letter to this affect is attached.

Marked up copies of the amended pages are attached.

An early and favorable Action on the merits is requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

B. J. Sadoff
Reg. No. **36,663**

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1992; Hierholzer et al., 1993; Fan and Hendricksom, 1996). The sequences of probes used for the others are 5'-CCT GCA TTA ACA CTA AAT TC-3' (SEQ ID NO 1) for RSV; 5'-TCT TGC TAC CTT CTG TAC TAA-3' (SEQ ID NO 2) for *C. pneumonia* and 5'-AAA ATT CCA AAA GAG ACC GGC-3' (SEQ ID NO 3) for PIV-3. All capture probes were 3'-biotinylated and purchased by Eurogentec (Seraing, Belgium), Capture was allowed to proceed for 1 h at 37°C, and afterwards the wells were washed four times with 200 µl of washing solution (Boehringer Mannheim, Mannheim, Germany) at room temperature. To each well 200 µl of anti-DIG-peroxidase (10 mU/ml, Boehringer Mannheim, Mannheim, Germany) diluted 1/1,000 in a buffer containing 100 mM Tris-HCl, 150 mM NaCl (pH 7.5) was added. Plates were incubated for 30 min at 37°C and wells were washed four times with washing solution. 200 µl ABTS® substrate solution (Boehringer Mannheim, Mannheim, Germany) was added, and the wells were incubated for 30 min at 37°C. The optical density (OD) was read on a DIAS reader (Dynatech Laboratories, Guernsey, Channel Islands) at 405 nm (reference filter 492 nm). The run was considered valid, if all negative control values were less than 0.2 OD units and the positive control was higher than 1.0 OD units. Samples were classified as PCR positive or negative according to a cut-off OD value of 0.5 and by comparison with the results from gel electrophoresis. Samples with initial readings of between 0.2 and 0.5 were considered borderline and were classified as positive or negative only after retesting with the specific single primer set. Positive hybridization controls were included in each microwell hybridization assay. They consisted of PCR products derived from the positive controls that were included in the m-RT-PCR.

7. Administration of data

All data obtained were managed in a Microsoft Access database. This database included all available information about patients as well as all diagnostic data and results from m-RT-PCR-ELISA, and in case of influenza A and RSV the data of the EIA.

8. Bacterial and viral stocks

Bacterial and viral stocks used as positive control were kindly provided by the following persons: B. Schweiger and E. Schreier, (Robert-Koch-Institute, Berlin) enteroviruses, Influenza A and influenza B; K.M. Edwards (Vanderbilt University, Tennessee, USA) RSV, PIV-1, PIV-3; A. Strecker (Institute for Virology, Bochum) RSV-long, PIV-3; R. Krausse and P. Rautenberg (Institute for Medical Microbiology, Kiel), *M. pneumoniae*, *C. pneumoniae*, and adenoviruses.

The exact number of viruses or bacteria within these samples was not known and was assumed to be at most 10^8/ml as stated by B. Schweiger and P. Rautenberg (personal

Table 1: Comparison of EIA and m-RT-PCR-ELISA

RSV		**EIA**	
		Pos.	Neg.
PCR	Pos.	116	25
	Neg.	24	866
	Total	140	891

InfA		**EIA**	
		Pos.	Neg.
PCR	Pos.	52	14
	Neg.	1	873
	Total	53	887

Table 2. Primer sequences used in Example 1

ENTERO- FP1:	att gtc acc ata agc agc ca-3'	(SEQ ID NO:35)
ENTERO- RP1:	tcc tcc ggc ccc tga atg cg-3'	(SEQ ID NO:36)
MPN-FP1:	aag gac ctg caa ggg ttc gt-3'	(SEQ ID NO:37)
MPN-RP1:	ctc tag cca tta cct gct aa-3'	(SEQ ID NO:38)
INFLUA-FP1:	aag ggc ttt cac cga aga gg-3'	(SEQ ID NO:39)
INFLUA-RP1:	ccc att ctc att act gct tc-3'	(SEQ ID NO:40)
INFLUB-FP1:	atg gcc atc gga tcc tca ac-3'	(SEQ ID NO:41)
INFLUB-RP1:	tgt cag cta tta tgg agc tg-3'	(SEQ ID NO:42)
ADENO-FP1:	gcc gag aag ggc gtg cgc agg ta-3'	(SEQ ID NO:43)
ADENO-RP1:	atg act ttt gag gtg gat ccc atg ga-3'	(SEQ ID NO:44)
CPN-FP1:	tga caa ctg tag aaa tac agc-3'	(SEQ ID NO:45)
CPN-RP1:	cgc ctc tct cct ata aat-3'	(SEQ ID NO:46)
PIV1-FP1:	cac atc ctt gag tga tta agt ttg atg a-3'	(SEQ ID NO:47)
PIV1-RP1:	att tct gga gat gtc ccg tag gag aac-3'	(SEQ ID NO:48)
PIV3-FP1:	tag cag tat tga agt tgg ca-3'	(SEQ ID NO:49)
PIV3-RP1:	aga ggt caa tac caa caa cta-3'	(SEQ ID NO:50)
RSV-FP1:	tgt tat agg cat atc att ga-3'	(SEQ ID NO:51)
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Table 3.

Organism	Original probe	Adapted versions*	Name**
Enterovirus	(SEQ ID NO:4) gaaacacggacacccaaagta	gaaacacggacacccaaagta (SEQ ID NO 4)	**entero1**
Influenza A	(SEQ ID NO:53) gtcctcatcggaggacttgaatggaatgat	catcggaggacttgaatgg (SEQ ID NO 5)	**influa1**
Influenza B	(SEQ ID NO:6) gtcaagagcaccgattatcac	gtcaagagcaccgattatcac (SEQ ID NO 6)	**influb1**
Adenovirus	(SEQ ID NO:54) ctcgatgacgccgcggtgc	gatgacgccgcggtg (SEQ ID NO 7)	adeno1
		tctcgatgacgccgcg (SEQ ID NO 8)	**adeno2**
		cataaagaagggtgggc (SEQ ID NO 9)	**adeno3**
Parainfluenza 1	(SEQ ID NO:55) taccttcattatcaattggtaagtcaatatatg	ccttcattatcaattggtaagtc (SEQ ID NO 10)	piv11
		ccttcattatcaattggtgatgc (SEQ ID NO 11)	**piv12**
		gttagaytaccttcattatcaattggt (SEQ ID NO 12)	piv13
Parainfluenza 3	(SEQ ID NO:13) aaaattccaaaagagaccggc	aaaattccaaaagagaccggc (SEQ ID NO 13)	**piv31**
RSV	(SEQ ID NO:1) cctgcattaacactaaattc	cacctgcattaacactaaattct (SEQ ID NO 14)	**rsv1**
M. pneumoniae	(SEQ ID NO:56) actcctacgggaggcagcagta	ctacgggaggcagcagt (SEQ ID NO 15)	**mpn1**
C. pneumoniae	(SEQ ID NO:16) tcttgctaccttctgtactaa	tcttgctaccttctgtactaa (SEQ ID NO 16)	**cpn1**

* y = c or t

** Probes in bold were used on first generation LiPA assay.

1992; Hierholzer et al., 1993; Fan and Hendricksom, 1996). The sequences of probes used for the others are 5'-CCT GCA TTA ACA CTA AAT TC-3' (SEQ ID NO 1) for RSV; 5'-TCT TGC TAC CTT CTG TAC TAA-3' (SEQ ID NO 2) for *C. pneumonia* and 5'-AAA ATT CCA AAA GAG ACC GGC-3' (SEQ ID NO 3) for PIV-3. All capture probes were 3'-biotinylated and purchased by Eurogentec (Seraing, Belgium), Capture was allowed to proceed for 1 h at 37°C, and afterwards the wells were washed four times with 200 µl of washing solution (Boehringer Mannheim, Mannheim, Germany) at room temperature. To each well 200 µl of anti-DIG-peroxidase (10 mU/ml, Boehringer Mannheim, Mannheim, Germany) diluted 1/1,000 in a buffer containing 100 mM Tris-HCl, 150 mM NaCl (pH 7.5) was added. Plates were incubated for 30 min at 37°C and wells were washed four times with washing solution. 200 µl ABTS® substrate solution (Boehringer Mannheim, Mannheim, Germany) was added, and the wells were incubated for 30 min at 37°C. The optical density (OD) was read on a DIAS reader (Dynatech Laboratories, Guernsey, Channel Islands) at 405 nm (reference filter 492 nm). The run was considered valid, if all negative control values were less than 0.2 OD units and the positive control was higher than 1.0 OD units. Samples were classified as PCR positive or negative according to a cut-off OD value of 0.5 and by comparison with the results from gel electrophoresis. Samples with initial readings of between 0.2 and 0.5 were considered borderline and were classified as positive or negative only after retesting with the specific single primer set. Positive hybridization controls were included in each microwell hybridization assay. They consisted of PCR products derived from the positive controls that were included in the m-RT-PCR.

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ADENO-FP1: gcc gag aag ggc gtg cgc agg ta-3' (SEQ ID NO: 43)
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CPN-RP1: cgc ctc tct cct ata aat-3' (SEQ ID NO: 46)

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PIV1-RP1: att tct gga gat gtc ccg tag gag aac-3' (SEQ ID NO: 48)

PIV3-FP1: tag cag tat tga agt tgg ca-3' (SEQ ID NO: 49)
PIV3-RP1: aga ggt caa tac caa caa cta-3' (SEQ ID NO: 50)

RSV-FP1: tgt tat agg cat atc att ga-3' (SEQ ID NO: 51)
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